DEVELOPMENT AND EVALUATION OF *CENTELLA ASIATICA* POWDER BY FREEZE DRYING: COMPARISON OF EXTRACTION METHODS ON YIELD AND HYGROSCOPICITY

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Teknik-teknik novel dan pembangunan kaedah terbaharu telah membuka peluang untuk menghasilkan ekstrak sebatian bioaktif semulajadi yang sangat tinggi di samping potensinya untuk merawat dan mencegah pelbagai penyakit. Kajian ini membandingkan peratusan dapatan serbuk *Centella asiatica* yang diperoleh melalui proses pengeringan beku yang diekstrak terlebih dahulu secara akueus melalui kaedah ekstrak konvensional (rebusan dan rendaman) dan kaedah bukan konvensional seperti pengekstrakan bantuan ultrabunyi. Kestabilan fizikal serbuk kemudiannya diuji melalui ujian higroskopi. Ujian ini menggunakan teknik analisis serapan gravimetrik dengan sedikit modifikasi. Setiap sampel serbuk melalui pra-rawatan pada 45 °C, 10% RH diikuti dengan proses ekuilibrasi pada 25 °C, 80% RH dan seterusnya dikategorikan mengikut European Pharmacopeia. Pengekstrakan bantuan ultrabunyi menghasilkan dapatan serbuk terbanyak (11.65%) diikuti oleh rendaman (10.48%) dan rebusan (10.03%). Setelah 24 jam dianalisa, ketiga-tiga sampel diklasifikasikan sebagai sangat higroskopi dengan peratusan serapan air melebihi 15%. Peratusan serapan air tertinggi adalah melalui rebusan (71.31%), diikuti oleh rendaman (71.28%) dan pengekstrakan bantuan ultrabunyi (68.71%).

ABSTRACT

Novel techniques and the development of new methods has provided an opportunity to obtain highly purified natural bioactive compound extracts with potential for the treatment and prevention of various diseases. This research compares the percentage yield of *Centella asiatica* obtained through freeze drying that was aqueous-extracted earlier by conventional extraction method (decoction and infusion) and an advanced non-conventional extraction method; ultrasound-assisted extraction (UAE).The physical stability of the powder obtained was then tested by hygroscopicity test. The studies were conducted using gravimetric sorption analysis method with slight modification. Each of the powder samples was subjected to the pretreatment at 45 °C, 10% RH followed by equilibrating at 25 °C, 80% RH and individually categorized as per European Pharmacopeia. UAE generated the highest powder yield (11.65%) followed by infusion (10.48%) and decoction (10.03%). After 24 hours of hygroscopicity analysis, all three samples were found to be very hygroscopic since their respective percentage of water sorption was more than 15%. The highest percentage of water sorption was by decoction (71.31%), followed by infusion (71.28%) and lastly UAE (68.71%).

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LIST OF SYMBOLS

cm	Centimetre
°C	Degree Celsius
G	Gram
hr	Hour
kcal	Kilo Calories
kg	Kilogram
kHz	Kilo Hertz
MHz	Mega Hertz
m/*m*	Mass per Mass
µg	Microgram
µm	Micrometre
mg	Milligram
ml	Millilitre
mm	Millimetre
mm Hg	Millimetre Mercury
mTorr	MilliTorr
mol	Mole
%	Percentage
w/v	Weight per Volume
w/w	Weight per Weight

LIST OF ABBREVIATIONS

AE	Ascorbic acid Equivalent
T_c	Collapse Temperature
E Ex	Ethanol Extract
Ph. Eur.	European Pharmacopeia
T_e	Eutectic Temperature
ES	Extractable Solids
GAE	Gallic Acid Equivalent
GAG	Glycosaminoglycan
M_{GSA}	Mass Gravimetric Sorption Analysis
M_{PT}	Mass Pre-treatment
PE	Pyrogallol Equivalent
QE	Quercetin Equivalent
RH	Relative Humidity
TE	Tannic acid Equivalent
TAC	Total Antioxidant Capacities
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TE	Trolox Equivalent
UAE	Ultrasound-Assisted Extraction
VH	Very Hygroscopic
H_2O Ex	Water Extract
XOI	Xanthine Oxidase Inhibitory

CHAPTER 1

INTRODUCTION

1.1 Background Study

Centella asiatica as shown in Figure 1.1, commonly known as centella, Asiatic pennywort or Gotu kola, is a herbaceous, frost-tender perennial plant belonging to the family Apiaceae. Easily grow and found in the most tropical and subtropical countries, *C. asiatica* is used for medicinal purposes (Singh and Singh, 2002). *C. asiatica* or Gotu kola should not be confused with kola nut as it does not contain any caffeine and has not been shown to have stimulant properties (Niamnuy et al., 2013). There are several important bioactive compounds present in *C. asiatica* such as triterpene saponins, phenolic compounds, vitamins, minerals, free amino acids, and polyacetylenic compounds (Niamnuy et al., 2013). All of these compounds are the major ingredients that are responsible for *C. asiatica* therapeutic effects.

Figure 1.1 *Centella Asiatica* (Source: Sudhakaran, 2017)

In the nineteenth century, *C. asiatica* and its extracts were incorporated into the Indian pharmacopoeia, wherein in addition to wound healing, it was recommended for various skin conditions treatment (Gohil, Patel and Gajjar, 2010; Gohil, Patel and Gajjar, 2012). *C. asiatica* extracts contain 70% triterpenic acids mainly two triterpene glycosides (asiaticoside and madecassoside) and corresponding aglycones (asiatic acid and madecassic acid) which exerts normalizing action on metabolism of connective tissues. It also enhances tissue integrity by stimulating glycosaminoglycan (GAG) synthesis without promoting excessive collagen synthesis or cell growth (Pizzorno, Murray and Joiner-Bey, 2016; Govarthanan et al., 2015). However, it is inconvenience for some users to consume it as it is. Thus, the *C. asiatica* plant is extracted before it is powdered and encapsulated to enhance its bioavailability, stability and shelf life (Rivas et al., 2017).

Extraction of botanicals and herbal sample is the crucial first step prior to analysis of bioactive compounds present in the preparations (Sasidharan et al., 2011). Various solvents can be used to extract the *C. asiatica* which includes methanol (Govarthanan et al., 2015), ethanol (Dewi and Maryani, 2015) and distilled water (Cheng et al., 2004). Different solvents will give different quantity of yield obtained. Due to unmatched availability in chemical diversity, pure compounds or standardized plant extracts provides unlimited opportunities for new drug discoveries (Cos et al., 2006). The extraction basic steps are pre-washing the sample, drying or freeze drying the plant material, grinding to obtain a homogenous sample and enhancing the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. During the extract preparation, proper actions must be taken to assure that there is no loss, distortion or denaturation of potential active constituents of the plant samples (Sasidharan et al., 2011).

Drying is a common method of preservation, applied by removing the water from the plant. Convection drying and freeze drying are the two most popular drying methods. Conventional drying such as oven drying is a user-friendly and very low cost method compared to freeze drying, which is a lengthy procedure and requires higher cost (Vashisth, Singh and Pegg, 2011). Previous research showed that there are major quality (nutrient compound and colour) differences of the final product after conventional drying compared to freeze dried products (Wojdylo et al., 2014).

Freeze drying is conducted to remove the solvent in such a way that the sensitive molecular structure of the active substance in the plant is least disturbed (Alexeenko, Ganguly and Nail, 2009). It is more suitable for products sensitive to temperature or to high residual moisture content (Touzet et al., 2018).

1.2 Problem Statement

The raw, unprocessed *C. asiatica* plant have a shorter shelf life and physically unstable. The leaves are easily wilted upon exposure to sunlight and its bioactive components degraded due to oxidation which leads to physical changes of the plant. *C. asiatica* fresh plant is very likely susceptible towards microbial growth due to high moisture content especially in leaves and stems (Brinkhaus et al., 2000). Besides that, it may be inconvenient for patient to consume it raw since some of them may not like to eat vegetables or herbs. This study is conducted to overcome this problem by converting the *C. asiatica* into powders. A study shows that *C. asiatica* lost a high amount of phenolic compounds (total phenolic content (TPC) and total flavonoid content (TFC) when extracted with ethanol of concentration 80% and above (Chew et al., 2011). In addition, the safety consumption of oral drug with active ingredients extracted using alcohol solvents is probably the main issue to deal with (Man and Choo, 2018). In other aspect, conventional extraction methods (maceration, decoction, infusion etc.) are the most convenient and inexpensive as it does not require any advanced equipment (Easmin et al., 2014). However, commercial extraction methods such as microwave-assisted extraction, ultrasound-assisted extraction and super critical fluid extraction have been proposed to extract bioactive compounds from plants with higher recovery capacity (Caldas et al., 2018). Hence, the solvent as well as the extraction methods used must be optimized to obtain the highest yield of *C. asiatica* powder that is physically more stable.

REFERENCES

Alexeenko, A.A., Ganguly, A., and Nail, S.L. (2009). Computational analysis of fluid dynamics in pharmaceutical freeze-drying. *Journal of Pharmaceutical Sciences*, Vol. 98. Issue 9. 3483-3494

Allada, R., Maruthapillai, A., Palanisamy, K., and Chappa, P. (2016). Hygroscopicity categorization of pharmaceutical solids by gravimetric sorption analysis: A systematic approach. *Asian Journal of Pharmaceutics*, Vol. 10. Issue 4. 279-286

Aramouni, F., and Deschenes, K. (2015). Methods for developing new food products. DEStech Publications Inc. 154-156

Atha, D. (2017). Hydrocotyle Sibthorpioides and H. Batrachium (Araliaceae) new for New York State. *Phytoneuro,* Vol. 56. 1–6

Belachew, T., and Lindstrom, D. (2015). Practical techniques of dehydration of foods. Koros Press Editorial Board. 85-88

Brinkhaus, B., Lindner, M., Schuppan, D., and Hahn, E. G. (2000). Chemical, pharmacological and clinical profile of the East Asian medical plant Centella Asiatica. *Phytomedicine,* Vol. 7(5). 427-448

Caldas, T. W., Mazza, K. E. L., Teles, A. S. C., Mattos, G. N., Brigida, A. I. S., Conte-Junior, C. A., Borguini, R. G., Godoy, R. L. O., Cabral, L. M. C., Tonon, R. V. (2018). Phenolic compounds recovery from grape skin using conventional and nonconventional extraction methods. *Industrial Crops & Products,* Vol. 111. 86-91

Callahan, J.C., Cleary, G.W., Elefant, M., Kaplan, G., Kensler, T., and Nash, R.A. (1982). Equilibrium moisture content of pharmaceutical excipients. *Drug Development and Industrial Pharmacy*, Vol. 8. Issue 3. 355-369

Castro-López, C., Ventura-Sobrevilla, J.M., González-Hernández, M.D., Rojas, R., Ascacio-Valdés, J.A., Aguilar, C.N., and Martínez-Ávila, G.C.G. (2017). Impact of extraction techniques on antioxidant capacities and phytochemical composition of polyphenol-rich extracts. *Food Chemistry*, Vol. 237. 1139-1148

Chemat, F., Rombaut, N., Sicaire, A-G., Meullemiestre, A., Fabiano-Tixier, A-S. , and AbertVian, M. (2016). Ultrasound assisted extraction of food and natural products. mechanisms, techniques, combinations, protocols and applications A Review. *Ultrasonics Sonochemistry*

Chen, D. (2009). Hygroscopicity of pharmaceutical crystals. Faculty of Graduate School of the University of Minnesota

Cheng, C. L., Guo, J. S., Luk, J., and Leung Koo, M. W. (2004). The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. *Life Sciences*, Vol. 74. Issue 18. 2237-2249

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., and Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Centella Asiatica extracts. *International Food Research Journal*, Vol. 18. 571-578

Cittan, M., Altuntas, E., and Celik, A. (2018). Evaluation of antioxidant capacities and phenolic profiles in Tilia cordata fruit extracts: A comparative study to determine the efficiency of traditional hot water infusion method. *Industrial Crops & Products,* Vol. 122, 553-558

Cong-Cong, X., Bing, W., Yi-Qiong, P., Jian-Sheng, T, and Tong, Z. (2017). Advances in extraction and analysis of phenolic compounds from plant materials. *Chinese Journal of Natural Medicines*, Vol. 15 (10). 721-731

Cos, P., Vlietinck, A.J., Berghe, D. V., and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology,* Vol. 106. Issue 3. 290-302

De Bruijn, J., Rivas, F., Rodriguez, Y., Loyola, C., Flores, A., Melin, P., and Borquez, R. (2016). Effect of vacuum microwave drying on the quality and storage stability of strawberries. *J. Food Process. Preservation*, Vol. 40. 1104-1115

Dent, M., Dragovic-Uzelac, V., Penic, M., Brncic, M., Bosiljkov, T and Levay, B. (2013). The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (Salvia officinalis L.) extracts. *Food Technology and Biotechnology*, Vol. 51. No 1. 84–91

Dewi, R.T., and Maryani, F. (2015). Antioxidant and α-glucosidase inhibitory compounds of Centella Asiatica. *Procedia Chemistry*, Vol. 17. 147-152

Easmin, MT. S., Sarker, M. Z. I., Ferdosh, S., Shamsudin, S. H., Yunus, K., Uddin, M. S., Sarker, M. M. R., Akanda, M. J. H., Hossain, M. S., and Khalil, HSP. A. (2014). Bioactive compounds and advanced processing technology: Phaleria macrocarpa (sheff.) Boerl, A review. *Journal of Chemical Technology & Biotechnology*

Franks, F.(2008). Freeze-drying of pharmaceuticals and biopharmaceuticals: principles and practice. Royal Society of Chemistry Publishing. 105-128

Gohil K.J., Patel J.A., Gajjar A.K. (2012). A pharmacological investigation of the potential herbal drug interaction between Centella Asiatica and imipramine in rats and mice. *Asian Pacific Journal of Tropical Biomedicine*. 1-11

Gohil K.J., Patel J.A., Gajjar A.K.. (2010). Pharmacological review on Centella asiatica: A potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences,* Vol. 72. No 5. 546-556

Govarthanan, M., Rajinikanth, R., Kamala-Kannan, S., and Selvankumar, T. (2015). A comparative study on bioactive constituents between wild and in vitro propagated Centella Asiatica. *Journal of Genetic Engineering and Biotechnology,* Vol. 13. Issue 1. 25-29

Joesting, H. M., Sprague, M. O., and Smith W. K. (2012). Seasonal and diurnal leaf orientation, bifacial sunlight incident, and leaf structure in the sand dune herb *Hydrocotyle Bonariensis*. *Environmental and Experimental Botany,* Vol. 75. 195-203

Karathanos, V. T., Anglea, S. A., and Karel, M. (1996). Structural collapse of plant materials during freeze-drying. *Journal of Thermal Analysis,* Vol. 47. Issue 5. 1451–1461

Katalinic, V., Milos, M., Kulisic, T., and Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry,* Vol. *94*. 550-557

Khatib, A., H, M., Shaari, K., Abas, F., Shitan, M., Kneer, R., Neto, V., and Lajis, H.N.(2012). Discrimination of three pegaga (Centella) varieties and determination of growth-lighting effects on metabolites content based on the chemometry of 1h nuclear magnetic resonance spectroscopy. *Journal of Agricultural and Food Chemistry*, Vol. 60. No 1 . 410-417

Labconco. (2004). A guide to freeze drying for the laboratory. Retrieved from http://www.thermofishersci.in/lit/Labconco%20-%20A%20laboratory%20guide%20to%20freeze%20drying.pdf

Lemus-Mondaca, R., Vega-Galvez, A., Rojas, P., Stucken, K., Delporte, C., Valenzuela-Barra, G., Jagus, R. J., and Aguero, M. V. (2018). Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods. *Journal of Applied Research on Medicinal and Aromatic Plants*

Levi, G., and Karel, M. (1995). Volumetric shrinkage (collapse) in freeze-dried carbohydrates above their glass transition temperature. *Food Research International*, Vol. 28. Issue 2. 145-151

Llyod, P.J., and Wyk, J.V. (2012). Enhancing extraction processes in the food industry. CRC Press. 1-22

LucidCentral. (2018). Herbal plant. Retrieved from https://keys.lucidcentral.org/keys/v3/bonsai/en/key/weeds_in_bonsai_en/Media/Html/desc_Hydrocotyle_sibthorpioides_var_sibthorpioides.html)

Ma, Q., Santhanam, R. K., Xue, Z., Guo, Q., Gao, X., and Chen, H. (2018). Effect of different drying methods on the physicochemical properties and antioxidant activities of mulberry leaves polysaccharides. *International Journal of Biological Macromolecules*

Malaysia Meteorological Department. (n.d). Maklumat ramalan cuaca. Retrieved from http://www.met.gov.my/home

Man, F., and Choo, C.Y. (2018). Safety assessment of standardized aqueous *Brucea Javanica* extract in rats. *Journal of Ethnopharmacology*, Vol. 215. 21-26

Maulidiani, M., Abas, F., Khatib, A., Shaari, K., and Lajis, N.H. (2014). Chemical characterization and antioxidant activity of three medicinal apiaceae species. *Industrial Crops and Products*, Vol. 55. 238–247

Newman, A.W., Reutzel-Edens, S.M. and Zografi, G. (2008). Characterization of the “hygroscopic” properties of active pharmaceutical ingredients. *Journal of Pharmaceutical Sciences*, Vol. 97. No 3. 1047-1059

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D., and Vuong, Q. V. (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. *Journal of Food Quality 2017*

Niamnuy, C., Charoenchaitrakool, M., Mayachiew, P., and Devahastin, S. (2013). Bioactive compounds and bioactivities of Centella asiatica (L.) Urban prepared by different drying methods and conditions. *International Journal of Drying Technology*, Vol. 31. Issue 16. 2007-2015

Oreopoulou, A., Tsimogiannis, D., and Oreopoulou, V. (n.d). Extraction of polyphenols from aromatic and medicinal plants: An overview of the methods and the effect of extraction parameters. *National Technical University of Athens*

Panja, P. (2017). Green extraction methods of food polyphenols from vegetable materials. *Food Science*, Vol. 10

Pferschy-Wenzig, E. M., and Bauer, R. (2015). The relevance of pharmacognosy in pharmacological research on herbal medicinal products. *Epilepsy & Behavior*,Vol. *52*. 344-362

Pizzorno, J. E., Murray, M. T., and Joiner-Bey, H. (2016). The clinician's handbook of natural medicine. Churchill Livingstone. Edition 3. 960-966

PlantNET. (1992). Hydrocotyle bonariensis Lam. Retrieved from http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/NSWfl.pl?page=nswfl&lvl=sp&name=Hydrocotyle~bonariensis

Plengmuankhae, W., and Tantitadapitak, C. (2015). Low temperature and water dehydration increase the levels of asiaticoside and madecassoside in Centella Asiatica (L.) Urban. *South African Journal of Botany*, Vol. 97. 196-203

Prosapio, V., Norton, I., and De Marco, I. (2017). Optimization of freeze-drying using a life cycle assessment approach: strawberries' case study. *Journal of Cleaner Production*, Vol. 168. 1171-1179

Pyper, J. W. (1985). The determination of moisture in solids: A selected review. *Analytica Chimica Acta*, Vol. 170. 159-175

Rahman, M., Hossain, S., Rahaman, A., Fatima, N., Nahar, T., Uddin, B., and Ahmed Basunia, M. (2013). Antioxidant activity of Centella asiatica (Linn.) Urban: impact of extraction solvent polarity. *Journal of Pharmacognosy and Phytochemistry*, Vol. 1. Issue 6

Ratti, C. (2001). Hot air and freeze-drying of high-value foods: a review. *Journal of Food Engineering*, Vol. 49. Issue 4. 311-319

Rivas, C. J. M., Tarhini, M., Badri, W., Miladi, K., Greige-Gerges, H., Nazari, Q. A., Rodríguez, S. A. G., Román, R. A., Fessi, H., and Elaissari, A. (2017). Nanoprecipitation process: from encapsulation to drug delivery. *International Journal of Pharmaceutics*, Vol. 532. Issue 1. 66-81

Ruenroengklin, N., Zhong, J., Duan, X., Yang, B., Li, J., and Jiang, Y. (2008). Effects of various temperatures and pH values on the extraction yield of phenolics from litchi fruit pericarp tissue and the antioxidant activity of the extracted anthocyanins. *International Journal of Molecular Sciences*, Vol. 9. No 7. 1333-1341

Sabaragamuwa, R., Perera, C. O., and Fedrizzi, B. (2018). Centella Asiatica (Gotu Kola) as a neuroprotectant and its potential role in healthy ageing. *Trends in Food Science & Technology*, Vol. 79. 88-97

SADC: South African Development Community. (2004). SADC guideline for stability testing. Retrieved from http://www.ich.org/fileadmin/Public_Web_Site/ABOUT_ICH/Organisation/SADC/Guideline_for_Stability_Studies.pdf

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., and Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plants extracts. *Afr J Tradit Complement Altern Med*, Vol. 8. No 1. 1–10

SEPPIC. (n.d). Challenges and solutions for moisture sensitive API formulation. Retrieved from https://www.seppic.com/pharmaceuticals/ingredients

Singh, P., and Singh, J. S. (2002). Recruitment and competitive interaction between ramets and seedlings in a perennial medicinal herb, Centella Asiatica. *Basic and Applied Ecology*, Vol. 3. Issue 1. 65-76

Stanisavljevic, D.M., Dordevic, S.M., Ristic, M.S., Velickovic, D.T., and Randelovic, N.V. (2010). Effects of different drying methods on the yield and the composition of essential oil from herb Mentha Longifolia (L.) Hudso. *Biologica Nyssana*, Vol. 1. No 1-2. 89-93

Sudhakaran, M. V. (2017). Botanical pharmacognosy of Centella asiatica (Linn.)Urban. *Pharmacognosy Journal*, Vol. 9(4). 546-558

Tan, S.H. (2010). Production of flavonoids in pegaga (Centella Asiatica L. Urban) cell suspension culture. PhD thesis, Universiti Putra Malaysia.

Touzet, A., Pfefferléa, F., Wel, P.V.D., Lamprecht, A., and Pellequer, Y. (2018). Active freeze drying for production of nanocrystal-based powder: A pilot study. *International Journal of Pharmaceutics*, Vol. 536. Issue 1. 222-230

USDA-NRCS: United States Department of agriculture-natural resources convention service. (n.d) Hydrocotyle Bonariensis Comm. ex Lam. Retrieved from https://plants.usda.gov/core/profile?symbol=hybo

Vashisth, T., Singh, R.K., and Pegg, R.B. (2011). Effects of drying on the phenolics content and antioxidant activity of Muscadine Pomace. *LWT - Food Science and Technology*, Vol. 44. Issue 7. 1649-1657

VirTis SP Scientific. (2014). BenchTop pro with Omnitronics-8L: freeze dryer operator's manual

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., and Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa Oleifera leaf extract by the appropriate extraction method. *Industrial Crops and Products*, Vol. 44. 566-571.

Wang, L. and Weller, C.L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends In Food Science & Technology,* Vol. 17. 300-312

Wojdylo, A., Figiel, A., Lech, K., Nowicka, P., and Oszmiański, J. (2014). Effect of convective and vacuum–microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. *Food and Bioprocess Technology*, Vol. 7. Issue 3. 829-841

Yahya, N.A., Attan, N. and Abdul Wahab, R. (2018). An overview of cosmeceutically relevant plant extracts and strategies for extraction of plant-based bioactive compounds. *Food and Bioproducts Processing,* Vol. 112. 69-85

Zainol, M.M.K., Abdul-Hamid, A., Abu Bakar, F., and Pak Dek, S. (2009). Effect of different drying methods on the degradation of selected flavonoids in Centella Asiatica. *International Food Research Journal*, Vol. 16. 531-537

Zainol, N.A., Voo, S.C., Sarmidi, M.R., and Aziz, R.A. (2008). Profiling of Centella Asiatica (L.) Urban Extract . *The Malaysian Journal of Analytical Sciences*, Vol. 12. No 2. 322 -327